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TITLE: Pilot Comparison of Stromal Gene Expression among Normal Prostate Tissues and Primary Prostate Cancer Tissues in White and Black Men

PRINCIPAL INVESTIGATOR: G. Steven Bova M.D.

CONTRACTING ORGANIZATION: Johns Hopkins University School of Medicine
                           Baltimore MD 21231

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G. Steven Bova M.D.

Email: gbov@jhmi.edu

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Johns Hopkins University School of Medicine
Baltimore MD 21231

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14. ABSTRACT

This hypothesis development project tests the feasibility of identification, laser capture microdissection, and expression analysis of prostate-stroma specific cells in normal and cancerous prostates, and aims to develop preliminary data sufficient to identify potential differences in stromal RNA expression in normal and cancerous prostate tissue. Our studies found that it is difficult but not impossible to histologically identify prostate zones with an acceptable degree of confidence in frozen tissues, eliminating the need to attempt expression studies in fixed tissues with their attendant biases. Laser capture microdissection of stromal tissue was completed for 5 normal prostates from men across the age range and of the two racial groups studied, and from prostates from men of similar ages with adenocarcinoma identified distant from the area of dissection. High Quality RNA was isolated, and duplicate Affymetrix Plus 2.0 chip analysis was recently completed. RNA expression data analysis has just begun. Selective validation testing of these analyzed data is planned and will be the final step of this painstaking hypothesis development project.

15. SUBJECT TERMS
  Prostate Stromal Gene Expression

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Introduction

Recent advances in prostate biology suggest that stromal cells surrounding prostate epithelia may play a key role in permitting or stimulating epithelial cells to lose control and form precancerous and cancerous lesions. The goal and purpose of this Hypothesis Development project is to obtain preliminary data sufficient to begin to explore the role of prostate stromal cells in prostate carcinogenesis under conditions as rigorously controlled as current technology allows.
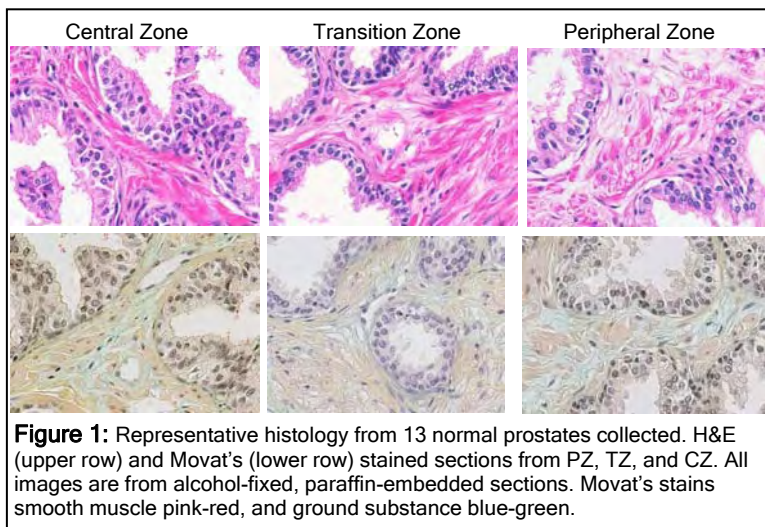
Body

Please note that because this is a Hypothesis Development Award project, no statement of work was required or provided as part of the original funded proposal. Nonetheless, the primary goal and steps required to complete the project were clearly identified in the original application.

As in any well-managed research project, we have kept our eyes on the goal, while changing tactics and methods to adjust to our own and outside research progress as the study progressed.

The following is a list of the main steps taken toward reaching the goal of this study:

1. Histologic analysis of available normal prostate sections from frozen and fixed (alcohol or formalin) tissue to identify tissue blocks which we can reliably be identified as containing prostate anatomic subzones (Central Zone, Peripheral Zone, Transition Zone, as illustrated in Figure 1). This was covered in our last study report. This was far more time-consuming than expected, but was completed successfully together with our prostate histology expert Dr. De Marzo.

Figure 1: Representative histology from 13 normal prostates collected. H&E (upper row) and Movat's (lower row) stained sections from PZ, TZ, and CZ. All images are from alcohol-fixed, paraffin-embedded sections. Movat's stains smooth muscle pink-red, and ground substance blue-green.

2. Decision to use only frozen tissues for RNA expression analysis for this pilot study based on discussion and careful review of current literature on using fixed tissues for RNA analysis. While many have reported the use of fixed tissues for RNA analysis, this method has limitations which should be avoided if sufficient frozen tissues are available. Analysis of the number and type of available tissues showed that we likely have sufficient frozen normal prostate for hypothesis development. If successful, this eliminates one layer of potential data confounding.

3. Continued work on improving function of our integrated database application to manage data emanating from the project. Our main focus has been on integrating RNA expression results with Tissue Microarray staining results. Because most current studies linking phenotype (cancer/normal for example) with molecular data (expression of specific gene products for example) do not have database control of all of their data, most expression studies to date report large amounts of data but do not stand up to recurrent hypothesis testing. With every new study performed in our laboratory, we attempt to improve these linkages to allow our science to continue to improve. This was covered in our previous progress report.

4. Decision to complete the study in collaboration with the Laboratory of Cancer Genetics, National Cancer Institute, headed by Dr. Michael Emmert-Buck. Our laboratories have a long history of collaboration, and our research interests are complementary. Together, we decided to widen the scope of the study to compare both epithelial and stromal RNA expression.

   Over the past several months, five of the normal cases identified above and five cases containing tumor foci elsewhere in the gland (from the Emmert-Buck laboratory) have been laser-capture microdissected for normal-appearing epithelium, and normal appearing stroma. RNA was then extracted using the PicoPure RNA Extraction Kit (Molecular Devices) and subjected to a quality and quantity control assessment. Quality of RNA was evaluated using an Agilent 2100 Bioanalyzer for RIN and 28S/28S ratios. Quantity was measured using a NanoDrop Spectrophotometer. Two rounds of linear oligo-dT amplification using the MessageAmp II Kit (Ambion) to amplify messenger RNA for GeneChip analysis. After a second Bioanalyzer step to confirm amplification, mRNA were fragmented and hybridized to oligo-nucleotide arrays (Affymetrix U133 Plus 2.0 GeneChips). The GeneChips contain over 54,000 probe sets and were run in technical replicates for quality assurance.

   Laser-capture microdissection went smoothly, with an average of 4,180 shots being taken for epithelial samples and 20,359 shots taken for stromal samples. A five-fold increase in shot number was necessary for stromal areas to increase RNA yield because it is a relatively cellular-poor region. Extracted RNA from each region averaged 13.49 ng/ul for the epithelium, and 8.33 ng/ul for the stromal samples. Quality assessment revealed 28S/18S ratios of 1.48 and 1.10 for the epithelium and stroma, respectively, and RINs (RNA Integrity Numbers) of 7.90 and 7.18, respectively as well. All of the gene chip hybridizations were completed as of Friday, January 19, 2007. Data analysis has begun this week and should be completed by the middle of February, at which point a candidate list of differentially-expressed transcripts will be available for validation. We will employ both immunostaining and real-time PCR for validation of the most impressive differences found. Results will be published in an appropriate journal and submitted to appropriate databases when completed in the next several months.

Key research accomplishments

- ✓ Identification of appropriate normal tissues for study
- ✓ Determination of frozen tissue as best foundation for study
- ✓ Completion of tedious laser capture microdissections
- ✓ Completion of Affymetrix Gene Chip Hybridizations

Reportable outcomes:

- ✓ None so far. Molecular Data analysis now beginning. Validation to be done next.

Conclusions:

✓ No major feasibility issues encountered in this hypothesis development project
✓ No molecular conclusions available yet, but will be available in next several months.

References: None so far

Appendices : None